Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 3816–3820, March 1998
Immunology

High affinity cross-reacting mAb generated by minimal mimicry: Implications for the pathogenesis of anti-nuclear autoantibodies and immunosuppression

ANNETTE L. ROTHERMEL AND DARIO C. ALTIERI*

Department of Pathology, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06536

Communicated by Frank J. Dixon, The Scripps Research Institute, La Jolla, CA, February 2, 1998 (received for review June 17, 1997)

ABSTRACT **The antigen recognition of a profoundly immunosuppressive mAb, mAb 2E1, *in vivo* was investigated. In addition to the 62-kDa effector cell protease receptor 1, mAb 2E1 bound the 32-kDa T cell adhesion receptor E2 (CD99) and the 86-kDa p80 subunit of the nuclear antigen complex Ku. These molecules share no overall sequence similarity. Peptide mapping experiments identified the mAb 2E1 cross-reacting epitopes as the sequences ^{66}GSFSDADLAD^{75} in E2 and ^{571}GGAHFSVSSLAEG^{583} in p80 of Ku, sharing a minimal homology motif FSXXXLA, in which X is a nonconserved amino acid. Each of these peptides separately inhibited the binding of mAb 2E1 to E2, effector cell protease receptor 1, and p80 of Ku in a dose-dependent manner. Scatchard plot analysis of ^{125}I-labeled mAb 2E1 binding to peripheral blood mononuclear cells revealed a high-affinity interaction with a dissociation constant of 7×10^{-10} M. An anti-E2 mAb bound the same epitope ^{66}GSFSDADLAD^{75} recognized by mAb 2E1 but failed to react with p80 of Ku and was not immunosuppressive. These findings demonstrate that high-affinity cross-reacting mAbs can be generated by mimicry of a minimal surface on unrelated molecules. This model of minimal mimicry may determine the nuclear reactivity of certain autoantibodies to Ku and contribute to aberrant immunosuppression *in vivo*.**

The development of a competent immune response *in vivo* depends on a "second signal" generated by costimulatory mechanisms (1) influencing lymphocyte activation and proliferation (2), cytokine gene expression (3), and inhibition of apoptosis (4). These mechanisms may also participate in aberrant activation of autoreactive T and B cells leading to T cell-mediated tissue damage (5) and production of pathogenic autoantibodies (6) in autoimmunity. In addition to the primary CD28/B7 system (2), alternative pathways of lymphocyte costimulation have been postulated from analysis of knockout animals (7, 8) and from probing the immune response with mAbs to T cell/monocyte surface molecules (9–11).

A potential alternative costimulatory mechanism was recently unveiled by the ability of a mAb, designated 2E1, to inhibit T cell activation and proliferation, blocking Ig production, cytokine release, and graft versus host disease, *in vivo* (12). This mAb was raised against viable T lymphoblastoid cells and selected for its reactivity with affinity-purified effector cell protease receptor 1 (EPR-1) (13), thus suggesting a potential role for this molecule in alternative lymphocyte costimulation (14). In this article, we report that mAb 2E1 recognizes three distinct antigens with high affinity, including p80 of Ku, a frequent nuclear target of autoantibodies (15, 16). These molecules lack overall sequence similarity and their cross-reacting epitopes contain a minimal homology motif FSXXXLA, in which X is a nonconserved amino acid. The potential implications of these findings for the generation of cross-reacting anti-nuclear autoantibodies and immunosuppression *in vivo* are discussed.

MATERIALS AND METHODS

mAbs and Synthetic Peptides. The establishment of mAb 2E1 has been described (13). Briefly, murine hybridomas were generated by i.p. injections of 10^6 viable EPR-1$^+$ MOLT13 T cells and screened for reactivity with MOLT13 cells by flow cytometry and with 62-kDa affinity-purified EPR-1 in Western blots (13). Seven positive mAbs were isolated and cloned twice by limiting dilution, and one of them (mAb 2E1, IgG2a) was used in immunologic screening of expression phage libraries to isolate the EPR-1 cDNA (13). Anti-p80 of Ku mAb D_6D_8 (17) was provided by M. Yaneva (Washington University, St. Louis, MO). Anti-E2 mAbs 12E7 (18) or 0662, L129, and D44 (19) were provided by R. Levy (Stanford University, Stanford, CA) and A. Bernard (Institut National de la Santé et de la Recherche Médicale, Nice, France). Sequential overlapping peptides from the extracellular region of E2 (K^{17}–D^{100}) and the C terminus of p80 of Ku (P^{503}–I^{731}), which contains most autoantibody epitopes, were synthesized by the W. M. Keck Biotechnology Laboratories at Yale University and subjected to reverse-phase HPLC liquid chromatography and mass spectrometry.

Cells and Cell Cultures. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood drawn from normal healthy volunteers by differential centrifugation over Ficoll/Hypaque (Pharmacia). PBMCs (5×10^5 cells per ml) were incubated in 96-well tissue culture plates with increasing concentrations (0.32–5000 ng/ml) of mAb 2E1 or anti-E2 mAbs for 30 min at 37°C and cultivated with anti-CD3 mAb OKT3 (1 μg/ml) for 3 days at 37°C. Cells were pulse-labeled with [^{3}H]thymidine at 1 μCi per well for 16 h (1 Ci = 37 GBq) and radioactivity incorporated under the various conditions was quantitated in a scintillation counter. In other experiments, mAb 2E1 was preincubated with control, E2, or p80 of Ku peptides for 30 min at 4°C before addition to PBMCs and determination of cell proliferation (12). The B lymphoma cell lines Daudi and Raji; monocytic cell line THP-1; erythroleukemia cell lines HEL, T leukemia cell lines MLT, Jurkat, and MOLT13; and epithelial cell line HeLa were obtained from the American Type Culture Collection and maintained in culture according to the supplier's specifications. The EPR-1 extracellular sequence M^1–R^{60}, containing the mAb 2E1 epitope, was engineered in the frame of intercellular adhesion molecule

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EPR-1, effector cell protease receptor 1; PBMC, peripheral blood mononuclear cell.

*To whom reprint requests should be addressed at: Yale University School of Medicine, BCMM 436B, 295 Congress Avenue, New Haven, CT 06536. e-mail: Dario.Altieri@yale.edu.

1 and the chimeric construct was transfected in Chinese hamster ovary cells by electroporation (20).

Affinity Chromatography, Immunoblotting, and Immunoprecipitation. MOLT13 cells (1×10^9 cells) were extracted in 0.15 M NaCl/0.05 M Tris·HCl/0.5% CHAPS (Calbiochem)/1 mM $CaCl_2$/1 mM PMSF, 1 mM benzamadine, 1 μM PPACK, 10 μg/ml leupeptin, and 10 μg/ml SBTI at pH 8.4, precleared, and applied to Affi-Gel (Bio-Rad)-coupled mAb 2E1 (27 mg) for 14 h at 4°C. After washes, mAb 2E1-bound material was eluted in 0.1 M glycine, pH 2.7/0.5% CHAPS and immediately neutralized in 1 M Tris·HCl (pH 9.6). Bands reactive with mAb 2E1 in immunoblotting (see below) were excised and microsequenced by using an Applied BioSystem gas-phase sequencer with on-line HPLC. For immunoblotting, mAb-2E1-affinity-purified material or detergent-solubilized cell extracts were separated on 7.5% or 10% SDS gels, and electroblotted to Immobilon (Millipore) at 450 mA for 2 h at 22°C. The transfer membrane was blocked with 5% nonfat dried milk, incubated with mAb 2E1 (20 μg/ml) for 2 h at 22°C, and then with ^{125}I-labeled goat anti-mouse $F(ab')_2$ fragments (Tago), followed by autoradiography.

Epitope Mapping. Ninety-six-well Immunolon II plates were coated with various peptides at 25 μg/ml for 16 h at 4°C, washed, blocked in 4% BSA, and incubated with control mouse IgG2a (Sigma), mAb 2E1, or various anti-E2 mAbs for 1 h at 37°C. After addition of biotin-conjugated goat anti-mouse IgG and alkaline phosphatase-conjugated streptavidin, binding of the primary mAbs was revealed with *p*-nitrophenyl phosphate (Zymed) at 1 mg/ml in 0.1 M 2-amino-2-propanediol (pH 10.3) and quantitated by absorbance at 405 nm.

Binding Studies and Immunofluorescence. PBMC, Jurkat, or EPR-1 transfectants (2×10^6 cells per ml) were preincubated with 20% normal human serum to prevent Fc-mediated mAb binding. Cells were sequentially incubated with control IgG2a (20 μg/ml) or other primary mAbs (20 μg/ml) and then with fluorescein isothiocyanate-conjugated goat anti-mouse $F(ab')_2$ (Biosource International, Camarillo, CA) for 30 min at 4°C and analyzed by flow cytometry. In other experiments, 2 mg of mAb 2E1 was radiolabeled with [^{125}I]NaI (Amersham) by the Iodo-Gen method to a specific activity of 8.7×10^5 cpm/μg of protein and free radioactivity was separated from protein-bound radioactivity by chromatography on a Sephadex G-25 PD-10 column (Pharmacia). For binding experiments, increasing concentrations of ^{125}I-labeled mAb 2E1 (0.01–16 μg/ml) were incubated with freshly isolated PBMCs (2×10^7 cells per ml) in serum-free RPMI 1640 medium for 30 min at 22°C, before separation of free and cell-surface bound radioactivity by centrifugation through a mixture of silicone oil. Nonspecific binding was assessed in the presence of a 50-fold molar excess of unlabeled mAb 2E1 or control anti-CD3 mAb OKT3 and was subtracted from the total to calculate specific binding. In peptide competition experiments, fluorescein isothiocyanate-conjugated mAb 2E1 was incubated with increasing concentrations of control or E2 or p80 of Ku peptides for 30 min at 22°C before determination of mAb binding to EPR-1$^-$ Jurkat cells (2×10^6 cells per ml) by flow cytometry. In other experiments, HeLa cells were grown on glass coverslips, fixed in 1% paraformaldehyde (Polysciences), and permeabilized with 0.5% Triton X-100 for 5 min at 22°C. After blocking with 4% goat serum, the coverslips were incubated with control IgG2a (5 μg/ml), mAb D_6D_8 (5 μg/ml), or mAb 2E1 (5 μg/ml) for 30 min at 22°C, followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Biosource International). After washes, cells were analyzed on a Nikon Microphot FXA (Nikon) fluorescence microscope. In some experiments, mAb 2E1 (5 μg/ml) was preincubated with 10 μM control peptide or E2 P^{59}–D^{75} or p80 of Ku G^{571}–E^{595} for 20 min at 22°C, before addition to permeabilized HeLa cells and determination of nuclear staining by immunofluorescence.

RESULTS

Anti-EPR-1 mAb 2E1 Recognizes Three Molecules. In immunoblots of detergent-solubilized cell extracts, mAb 2E1 reacted with three distinct molecules of 32, 62, and 86 kDa (Fig. 1*A*). The fainter 62-kDa band was consistent with the size of EPR-1 (13) and was more prominently detected in OKT3-stimulated PBMCs (Fig. 1*A*), in agreement with the activation-dependent expression of EPR-1 on these cells (14). With the exception of Daudi (see below and ref. 19), the two additional bands of 32 and 86 kDa recognized by mAb 2E1 were ubiquitously found in all cells types examined (Fig. 1*A*). Indistinguishable results were obtained with all seven mAbs of the 2E1 panel originally selected for reactivity with affinity-purified EPR-1 (13). In parallel experiments, mAb 2E1 immunoprecipitated all three bands of 32, 62, and 86 kDa from metabolically labeled cell extracts, whereas only the 32- and 62-kDa bands could be resolved from ^{125}I-surface-labeled cell extracts (data not shown). Fractionation of MOLT13 extracts on a mAb 2E1 affinity column yielded three distinct bands of 32, 62, and 86 kDa (Fig. 1*B*). By microsequencing, the 32-kDa band corresponded to the T cell surface adhesion receptor E2 (CD99) (19) (Fig. 1*B*), and immunologic screening of a λgt11 T cell MLT library with mAb 2E1 yielded an immunoreactive clone (λ101) encoding the E2 sequence K^{17}–K^{129} (19). The

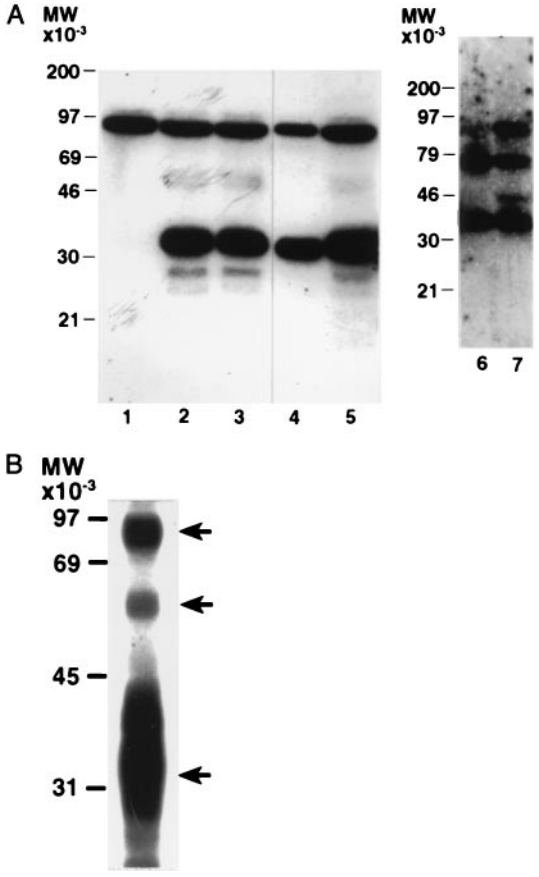

FIG. 1. Multiple antigenic reactivity of mAb 2E1. (*A*) Immunoblotting of detergent-solubilized cell extracts with mAb 2E1. Daudi (lane 1), HL-60 (lane 2), U937 (lane 3), human umbilical vein endothelial cells (lane 4), MOLT13 (lane 5), and 3-day (lane 6) or 7-day (lane 7) OKT3-activated PBMCs. (*B*) Affinity purification of mAb 2E1 immunoreactive material. Arrows indicate the position of the three molecules of 32, 62, and 86 kDa purified from MOLT13 cell extracts on a mAb 2E1-Affi-Gel column. The sequence of the 32-kDa band corresponded to the mature E2 N terminus APDGGFDLSDAL-PDNENKKP, whereas the 86-kDa band matched the p80 of Ku sequence VRSGNKAAVVLCMDVGFTMS. Relative molecular weight markers are indicated on the left.

sequence of the 86-kDa band matched the p80 subunit of the nuclear antigen complex Ku (21) (Fig. 1*B*), and mAb 2E1 strongly reacted with nuclei of permeabilized HeLa cells in immunofluorescence, indistinguishably from anti-p80 of Ku mAb D_6D_8 (see below). Although no interpretable sequence was obtained from the 62-kDa band (Fig. 1*B*), its identity as EPR-1 was confirmed by functional cloning of the cDNA with mAb 2E1 and by antibody reactivity with mammalian cells transfected with this cDNA (13).

Identification of the mAb 2E1 Cross-Reacting Epitopes on E2 and p80 of Ku. In peptide mapping experiments, mAb 2E1 bound a single sequence of P^{59}–D^{75} in E2 and G^{571}–E^{595} in p80 of Ku (Table 1). In contrast, none of the other E2- or p80 of Ku-derived sequences were recognized by mAb 2E1, under the same experimental conditions (Table 1). The mAb 2E1 cross-reacting epitopes were further narrowed to E2 ^{66}GSFSDADLAD^{75} and p80 of Ku ^{571}GGAHFSVSSLAEG^{583} (Table 1). The mAb 2E1 epitope on EPR-1 was previously mapped to M^1–R^{60} by using chimeric constructs expressed in mammalian cells (20).

Peptidyl Mimicry of mAb 2E1 Multiple Antigenic Reactivity. In ELISA, binding of mAb 2E1 to immobilized E2 or p80 of Ku epitopes was completely inhibited in a dose-dependent manner by either E2 P^{59}–D^{75} (IC_{50} range, 2–26 μM) or p80 of Ku G^{571}–E^{595} (IC_{50} range, 0.1–0.5 μM), whereas control Ku peptide G^{583}–E^{595} was ineffective. Similarly, each E2 or p80 of Ku epitope separately inhibited binding of mAb 2E1 to E2 on EPR-1$^-$ Jurkat T cells (Fig. 2*A*), blocked nuclear staining of p80 of Ku in permeabilized HeLa cells (Fig. 2*B*), and suppressed the antibody recognition of the EPR-1 epitope M^1–R^{60} on chimeric transfectants (Fig. 2*C*). In contrast, control E2 L^{73}–G^{89} or p80 of Ku I^{638}–Q^{662} (Table 1) were ineffective (Fig. 2). In direct binding experiments, ^{125}I-labeled mAb 2E1 associated with resting PBMCs in a specific and saturable reaction that was inhibited by >95% by a molar excess of unlabeled mAb 2E1 but not by control mAb OKT3. Scatchard plot analysis revealed a dissociation constant (K_d) of 7×10^{-10} M for mAb 2E1 binding to PBMCs.

Table 1. Epitope mapping of mAb 2E1 recognition of E2 and p80 of Ku

Sequence	A_{405}
E2 peptide	
^{17}KPTAIPKKPSAGDDFDL^{33}	0.051 ± 0.009
^{31}FDLGDAVVDGENDDPRP^{47}	0.073 ± 0.011
^{45}PRPPNPPKPMPNPNPNH^{61}	0.047 ± 0.002
^{59}PNHPSSSGSFSDADLAD^{75}	1.061 ± 0.062
^{73}LADGVSGGEGKGGSDGG^{89}	0.08 ± 0.005
^{87}DGGGSHRKEGEEAD^{100}	0.067 ± 0.01
^{56}NPNPNHPSSS^{65}	0.035 ± 0.001
^{66}GSFSDADLAD^{75}	0.427 ± 0.001
p80 of Ku peptide	
^{503}PLPPIQQHIWNMLNPPAEVTTKSQI^{527}	0.042 ± 0.003
^{525}SQIPLSKIKTLFPLIEAKKKDQVTA^{549}	0.037 ± 0.001
^{548}TAQEIFQDNHEDGPTAKKLKTEQGG^{572}	0.041 ± 0.001
^{571}GGAHFSVSSLAEGSVTSVGSVNPAE^{595}	0.365 ± 0.024
^{592}NPAENFRVLVKQKKASFEEASNQLI^{616}	0.057 ± 0.006
^{638}IRAFREEAIKFSEEQRFNNFLKALQ^{662}	0.055 ± 0.001
^{661}LQEKVEIKQLNHFWEIVVQDGITLI^{685}	0.057 ± 0.001
^{684}LITKEEASGSSVTAEEAKKFLAPKD^{708}	0.063 ± 0.001
^{707}KDKPSGDTAAVFEEGGDVDDLLDMI^{731}	0.07 ± 0.001
^{571}GGAHFSVSSLAEG^{583}	0.555 ± 0.11
^{583}GSVTSVGSVNPAE^{595}	0.039 ± 0.001

Ninety six-well plastic microtiter plates were coated with the indicated peptides from the extracellular region of E2 (K^{17}–D^{100}) or the C terminus of p80 of Ku (P^{503}–I^{731}) at 25 μg/ml. Wells were then coated with 4% BSA before determination of mAb 2E1 reactivity (5 μg/ml) by ELISA. Background absorbance in the presence of mouse IgG2a was less than an A_{405} value of ~0.04. Data are the mean ± SD for replicate wells of a representative experiment of three determinations.

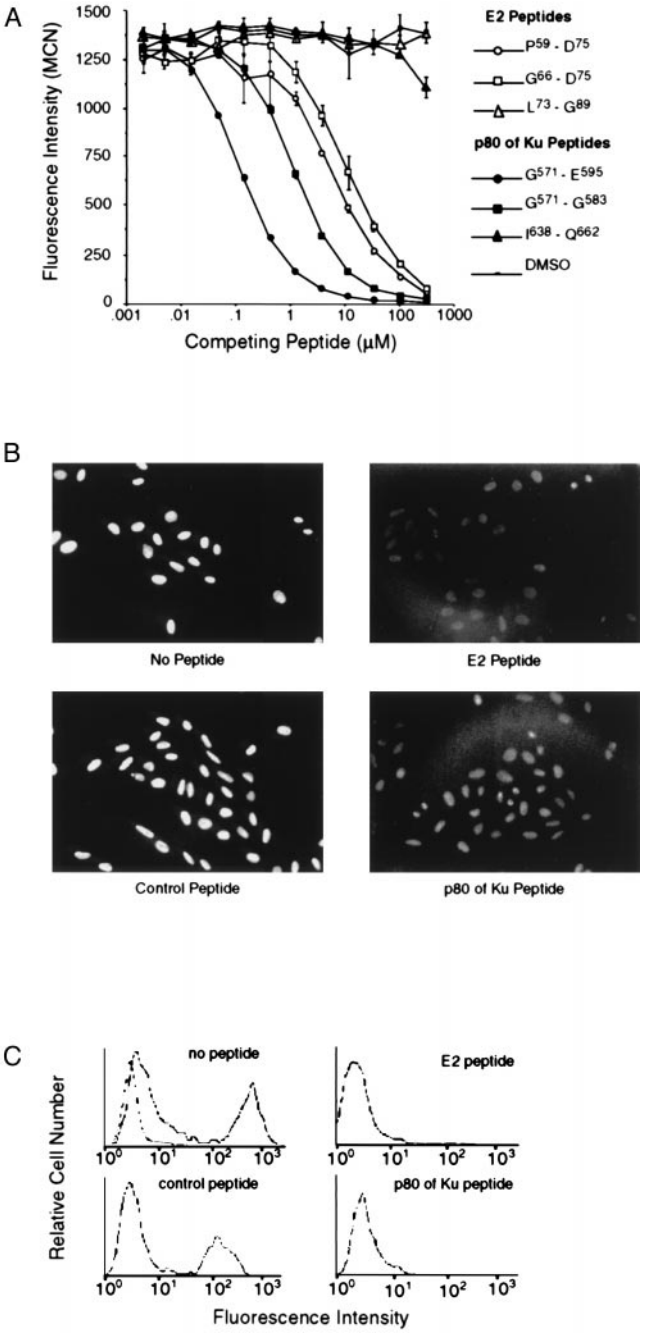

FIG. 2. Peptidyl mimicry of mAb 2E1 recognition of native E2, p80 of Ku, and EPR-1. (*A*) Peptide inhibition of mAb 2E1 reactivity with E2 on EPR-1$^-$ Jurkat T cells by flow cytometry. MCN, mean channel number. (*B*) Inhibition of mAb 2E1 nuclear staining of p80 of Ku by immunofluorescence. (*C*) Inhibition of mAb 2E1 reactivity with EPR-1 transfectants expressing the EPR-1 epitope M^1–R^{60} in the context of the intercellular cell adhesion molecule 1 chimeric frame by flow cytometry. The p80 of Ku peptide I^{638}–Q^{662} was used as a control. Data are representative of one experiment of at least three determinations.

Minimal Mimicry in the E2 Cross-Reacting Epitope. Epitope mapping of anti-E2 mAbs revealed that mAbs 0662 and L129 reacted with the same E2 sequence P^{59}–D^{75} recognized by mAbs 2E1 (Fig. 3*A*) and 0662 dose-dependently inhibited mAb 2E1 binding to E2$^+$ EPR-1$^-$ Jurkat T cells indistinguishably from unlabeled mAb 2E1 (data not shown). However, mAb 0662 and other anti-E2 mAbs failed to react

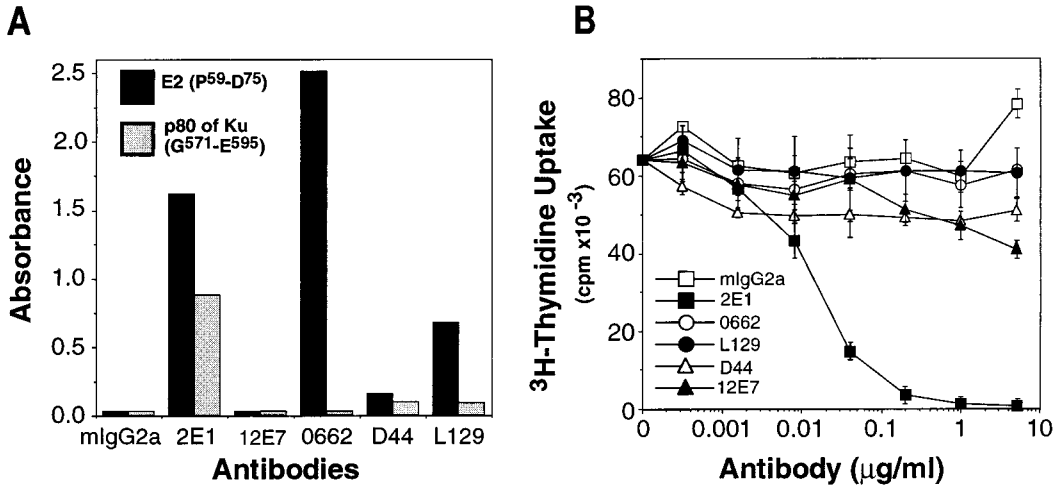

FIG. 3. Minimal mimicry of mAb 2E1 cross-reactivity with E2. (*A*) Epitope mapping of anti-E2 mAbs by ELISA. (*B*) Effect of anti-E2 mAbs or mAb 2E1 on CD3-dependent PBMC proliferation. Cell viability during the various culture conditions was always >97%. Data are representative of one experiment of three determinations.

with p80 of Ku peptides (Fig. 3*A*) and did not bind to EPR-1 transfectants by flow cytometry (data not shown). Similarly, anti-E2 mAbs, including mAb 0662, did not reduce mAb OKT3-stimulated PBMC proliferation at any concentration tested (Fig. 3*B*), which was dose-dependently inhibited by mAb 2E1 (Fig. 3*B*), in agreement with previous observations (12). Finally, injection of the E2 or p80 of Ku cross-reacting peptide epitopes in rabbits elicited the generation of monospecific antibodies, which did not cross-react with either molecule (data not shown).

DISCUSSION

In this study, we have shown that a potently immunosuppressive antibody *in vivo*, mAb 2E1 (12), recognizes three distinct molecules. These were identified by direct microsequencing of antibody affinity-purified material, functional cDNA cloning, and synthetic peptidyl mimicry as 62-kDa EPR-1 (13), 32-kDa E2 (CD99) (19), and 86-kDa p80 subunit of the nuclear antigen complex Ku (21). These molecules share no overall similarity and their cross-reacting epitopes, E2 ^{66}GSFSDADLAD^{75} and p80 of Ku ^{571}GGAHFSVSSLAEG^{583}, contain a minimal homology motif, FSXXXLA, in which X is a nonconserved amino acid.

This unusual pattern of antibody cross-reactivity is not due to accidental contamination of mAb 2E1 with unrelated hybridomas. (*i*) All seven mAbs independently selected for binding to isolated EPR-1 and cloned twice by limiting dilution (13) also reacted with E2 and p80 of Ku by immunoblotting and were immunosuppressive (unpublished observations). (*ii*) mAb 2E1 reactivity with E2, EPR-1, and p80 of Ku was completely cross-blocked by each peptide epitope separately and did not require peptide combinations. (*iii*) Indistinguishable results were obtained with culture supernatant or ascites-derived IgG fractions of mAb 2E1, thus ruling out a potential contamination of ascites fluid with unrelated murine antibodies *in vivo*.

There are several known mechanisms potentially mediating antibody cross-reactivity. In addition to low-affinity ($\sim 10^{-5}$ M) natural antibodies (22), cross-reacting antibodies can be generated by molecular mimicry of conserved, albeit not completely identical, amino acid sequences (23, 24), or by recognition of carbohydrate(s) determinants on unrelated glycoproteins (25). In our study, E2 and p80 of Ku peptides inhibited mAb 2E1 binding to their respective native macromolecules, thus excluding a role of shared carbohydrate epitopes in this cross-reactivity. Rather, these data suggest a mechanism of minimal mimicry, in which recognition of a limited surface area of similarity, potentially within the FSXXXLA homology motif, may be sufficient to determine high-affinity (i.e., $K_d = 7 \times 10^{-10}$ M) antibody cross-reactivity with E2, EPR-1, and p80 of Ku. It is plausible that other mechanisms may participate in this multiple antigen recognition because anti-E2 mAb 0662, which mapped to the same E2 sequence ^{66}GSFSDADLAD^{75} recognized by mAb 2E1, failed to cross-react with EPR-1 or p80 of Ku and to block T cell proliferation. Similarly, injection of the E2 or p80 of Ku peptide epitopes in rabbits elicited antiserum that did not cross-react with the immunizing sequences (unpublished observations). Although the fine molecular requirements of mAb 2E1 multiple recognition have not been elucidated, these findings point to an unusual degree of flexibility in antigen–antibody complementarity. Cross-reactivity between structurally unrelated molecules has been described for both antigen–antibody interactions (26–30) and other protein interactions (31–34). Structural studies assessing these cross-reactions have implicated conformational changes in the binding sites, involvement of similar bonding interactions or contact points, and solvation to increase complementarity (28, 29, 32, 33).

The paradigm of mAb 2E1 might have considerable implications for disease pathogenesis. Autoantibodies to both p70 and p80 of Ku are frequently found in patients with systemic lupus erythematosus and scleroderma–polymyositis overlap syndrome (15, 16). Autoantibodies are also a hallmark of infections with HIV or Epstein–Barr virus (35, 36) and primary effectors of tissue damage and disease progression (6). In this context, minimal mimicry of viral or self determinants during B cell activation may lead to the generation of high-affinity cross-reacting antibodies similar to mAb 2E1. Although this may explain the reactivity of certain autoantibodies with Ku (15, 16), it may also translate into profound immunosuppression *in vivo* (12). Consistent with this concept, bispecific autoantibodies reacting with nuclear and plasma membrane components have been previously described in autoimmune diseases (26, 27, 37, 38), and immunosuppressive autoantibodies have been shown to exacerbate HIV infection (39). Elucidation of the fine structural requirements of mAb 2E1 cross-reactivity should provide important insights on the pathogenic mechanisms of autoimmunity and immunosuppression, *in vivo*.

We thank Drs. M. Yaneva for discussion and for providing anti-Ku mAb D_6D_8, R. Levy for anti-E2 mAb 12E7, and A. Bernard for anti-E2 mAbs 0662, D44, and L129. The expert technical assistance of Ms. S.

Stamnes is gratefully acknowledged. We also thank Drs. P. Sanna for initial immunofluorescence studies and G. Ambrosini for EPR-1–intercellular cell adhesion molecule 1 chimeras. This work was supported by National Institutes of Health Grants HL43773 and HL54131. This work was done during the tenure of an American Heart Association Established Investigatorship Award to D.C.A.

1. Janeway, C. A., Jr., & Golstein, P. (1993) *Curr. Opin. Immunol.* **5,** 313–323.
2. Lenschow, D. J., Walunas, T. L. & Bluestone, J. A. (1996) *Annu. Rev. Immunol.* **14,** 233–258.
3. Gimmi, C. D., Freeman, G. J., Gribben, J. G., Sugita, K., Freedman, A. S., Morimoto, C. & Nadler, L. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6575–6579.
4. Boise, L. H., Minn, A. J., Noel, P. J., June, C. H., Accavitti, M. A., Lindsten, T. & Thompson, C. B. (1995) *Immunity* **3,** 87–98.
5. Zamvil, S. S. & Steinman, L. (1990) *Annu. Rev. Immunol.* **8,** 579–621.
6. Ray, S. K., Putterman, C. & Diamond, B. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2019–2024.
7. Shahinian, A., Pfeffer, K., Lee, K. P., Kundig, T. M., Kishihara, K., Wakeham, A., Kawai, K., Ohashi, P. S., Thompson, C. B. & Mak, T. W. (1993) *Science* **261,** 609–612.
8. Freeman, G. J., Borriello, F., Hodes, R. J., Reiser, H., Hatchcock, K. S., Laszlo, G., McKnight, A. J., Kim, J., Du, L., Lombard, D. B. *et al.* (1993) *Science* **262,** 907–909.
9. Cocks, B. J., Chang, C.-C. J., Carballido, J. M., Yssel, H., de Vries, J. E. & Aversa, G. (1995) *Nature (London)* **376,** 260–263.
10. Rothman, B. L., Blue, M.-L., Kelley, K. A., Wunderlich, D., Mierz, D. V. & Aune, T. M. (1991) *J. Immunol.* **147,** 2493–2499.
11. Reinhold, M. I., Lindberg, F. P., Kersh, G. J., Allen, P. M. & Brown, E. J. (1997) *J. Exp. Med.* **185,** 1–11.
12. Duchosal, M. A., Rothermel, A. L., McConahey, P. J., Dixon, F. J. & Altieri, D. C. (1996) *Nature (London)* **380,** 352–356.
13. Altieri, D. C. (1994) *J. Biol. Chem.* **269,** 3139–3142.
14. Altieri, D. C. (1995) *FASEB J.* **9,** 860–865.
15. Reeves, W. H., Pierani, A., Chou, C.-H., Ng, T., Nicastri, C., Roeder, R. G. & Sthoeger, Z. M. (1991) *J. Immunol.* **146,** 2678–2686.
16. Mimori, T., Hardin, J. A. & Steitz, J. A. (1986) *J. Biol. Chem.* **261,** 2274–2278.
17. Wen, J. & Yaneva, M. (1990) *Mol. Immunol.* **27,** 973–980.
18. Levy, R., Dilley, J., Fox, R. I. & Warnke, R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6552–6556.
19. Gelin, C., Aubrit, F., Phalipon, A., Raynal, B., Cole, S., Kaczorek, M. & Bernard, A. (1989) *EMBO J.* **8,** 3253–3259.
20. Ambrosini, G. & Altieri, D. C. (1996) *J. Biol. Chem.* **271,** 1243–1248.
21. Yaneva, M., Wen, J., Ayala, A. & Cook, R. (1989) *J. Biol. Chem.* **264,** 13407–13411.
22. Casali, P. & Notkins, A. L. (1989) *Immunol. Today* **10,** 364–368.
23. Oldstone, M. B. A. (1987) *Cell* **50,** 819–820.
24. Barnett, L. A. & Fujinami, R. S. (1992) *FASEB J.* **6,** 840–844.
25. Feizi, T. & Childs, R. A. (1987) *Biochem. J.* **245,** 1–11.
26. Jacob, L., Lety, M.-A., Bach, J.-F. & Louvard, D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6970–6974.
27. Madaio, M. P., Carlson, J., Cataldo, J., Ucci, A., Migliorini, P. & Pankewycz, O. (1987) *J. Immunol.* **138,** 2883–2889.
28. Fields, B. A., Goldbaum, F. A., Ysern, X., Poljak, R. J. & Mariuzza, R. A. (1995) *Nature (London)* **374,** 739–742.
29. Bentley, G. A., Boulot, G., Riottot, M. M. & Poljak, R. J. (1990) *Nature (London)* **348,** 254–257.
30. Savoca, R., Schwab, C. & Bosshard, H. R. (1991) *J. Immunol. Methods* **141,** 245–252.
31. Shoichet, B. K., Stroud, R. M., Santi, D. V., Kuntz, I. D. & Perry, K. M. (1993) *Science* **259,** 1445–1450.
32. Mol, C. D., Arvai, A. S., Sanderson, R. J., Slupphaug, G., Kavli, B., Krokan, H. E., Mosbaugh, D. W. & Tainer, J. A. (1995) *Cell* **82,** 701–708.
33. de Vos, A. M., Ultsch, M. & Kossiakoff, A. A. (1992) *Science* **255,** 306–312.
34. Wrighton, N. C., Farrell, F. X., Chang, R., Kashyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K. & Dower, W. J. (1996) *Science* **273,** 458–464.
35. Ditzel, H. J., Barbas, S. M., Barbas, C. F., III & Burton, D. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3710–3714.
36. Vaughan, J. H., Nguyen, M.-D., Valbracht, J. R., Patrick, K. & Rhodes, G. H. (1995) *J. Clin. Invest.* **95,** 1316–1327.
37. Searles, R. P., Messner, R. P. & Bankhurst, A. D. (1979) *Clin. Immunol. Immunopathol.* **14,** 292–299.
38. Rekvig, O. P. & Hannestad, K. (1980) *J. Exp. Med.* **152,** 1720–1733.
39. Golding, H., Shearer, G. M., Hillman, K., Lucas, P., Manischewitz, P., Zajac, R. A., Clerici, M., Gress, R. A., Boswell, R. N. & Golding, B. (1989) *J. Clin. Invest.* **83,** 1430–1432.